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# Determination of non-starch polysaccharides in cereal grains with near-infared reflectance spectroscopy

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Cereal grains contain variable amounts of non-starch polysaccharides, such as arabinoxylans and (1→3),(1→4)-β-glucans (β-glucans), which are associated with their cell walls. The type and composition of these polysaccharides is of increasing interest in both human and animal nutrition. Reference analysis for these polysaccharides requires the use both enzymic and monosaccharide methods. To evaluate fully the non-starch polysaccharides present in grains, some analysts further distinguish between the soluble and insoluble fractions of these components. Near-infrared reflectance (NIR) spectroscopy provides fast, inexpensive analysis. It is, however, a comparative technique that relies on multivariate calibration of sample spectra and accurate reference analysis. It has the potential to be exploited as a rapid analytical method for nutritionally important polysaccharides. The calibration statistics for arabinoxylans and β-glucans obtained in this study suggest that NIR can be used in plant breeding, nutritional and product studies to obtain simple and rapid estimates of non-starch polysaccharides. The occurrence of wheats with high cell wall contents together with barleys with high β-glucan contents is well known. However, to date, this genetic variation has not been extensively exploited for the production of grains for use as human food ingredients.

**Keywords:** Arabinoxylan / Cereal grains / Near-infrared reflectance spectroscopy / Non-starch polysaccharides

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## 1 Introduction

Cereal grains contain variable amounts of non-starch polysaccharides, such as arabinoxylans and (1→3),(1→4)-β-glucans (β-glucans), which are associated with their cell walls. The type and composition of these polysaccharides, as dietary fibre, is of increasing interest in both human and animal nutrition. A recent review has shown numerous interactions [1] and many of the advantages proposed for whole grains can be related to their increased non-starch polysaccharide content [2]. Conventionally, polysaccharide analysis had, in the past, been slow and expensive, so that a few samples were used to establish "typical values" for grain types. In the past 25 years, more modern gas or liquid chromatography-based methods have become widely used for monosaccharide analysis. Other methods based on the degradation of polysaccharides with specific enzymes have also been developed and many are now available as test kits. To evaluate fully the non-starch polysaccharides present in grains, some analysts further distinguish between soluble and insoluble fractions. Such a separation of non-starch polysaccharides is analytically difficult, but may be nutritionally significant.

The newer chemical and enzymic methods have led to much more specific analysis of grain non-starch polysaccharides. Total monosaccharide analysis [3, 4] and methylation analysis [5, 6] which can demonstrate the way in which individual sugars are linked together, was greatly simplified in the 1980s and many analytical programmes now routinely use these methods. The methodology is, however, still not fast or cheap enough to allow its use in the segregation of grain; it is still exacting, expensive, and requires special equipment. Enzyme-based test kits are also relatively slow, but use less specialised equipment.

If faster and cheaper analytical methods can be developed it would be much easier to identify grain samples with known concentrations of any of the non-starch polysaccharides. The methods could be used in nutritional studies, in studies

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**Abbreviations: NIR,** near-infrared reflectance; **NSP**, non-starch polysaccharide

to identify genetically superior grain lines, and in studies to determine the influence of climate or growing conditions on the concentrations of non-starch polysaccharides.

Near infrared reflectance (NIR) or transmission (NIT) spectroscopy has become the method of choice for the analysis of grain as it is received from farmers into marketing systems. It is also widely used in plant breeding programmes for the selection of cross-breeds with the desired qualities. NIR spectroscopy provides fast, safe, and inexpensive analysis. It is, however, a comparative technique that relies on multivariate calibration of sample spectra and accurate reference analysis [7, 8]. It has the potential to be exploited as a rapid analytical method for nutritionally important components, including polysaccharides. Over the past 25 years, the number of applications of NIR spectroscopy has grown rapidly, as demonstrated by the emergence of dedicated journals and large international conferences. In this paper, we present preliminary calibrations for arabinoxylans and β-glucans for a range of cereal grains and pulses. The data is taken from an Australian National feed grain project. All the grains included were selected to represent a wide genetic range and many were obtained from areas that had variable climatic conditions during crop growth.

## 2 Materials and methods

### 2.1 Materials

A large national research project in Australia has investigated the compositional and functional characteristics of grains and pulses likely to influence their nutritional value for livestock. NIR spectra and reference values for a wide range of chemical, physical, *in vitro* and *in vivo* measurements were obtained on a set of samples of grains and pulses with diverse genetic and environmental backgrounds. A total of 82 of these grains and pulses have been fed to animals and nutritional data collected. This set of samples, and a subset of them, was used for the NIR studies reported here. The full set contained 26 barleys, 18 wheats, 7 triticales, 12 oats, 9 sorghums, 1 maize, 4 lupins, 2 field peas, 2 chickpeas, and 1 faba bean.

### 2.2 NIR methodology

All grains and pulses were ground using a Newport 6200 cyclone mill (Newport Scientific, Warriwood, NSW, Australia) to pass a 1 mm screen. This mill has a similar grinding action to a Udy or Cyclotec mill but has a stainless steel impeller and diamond set in a nickel grinding ring that results in very little contamination of the sample [9]. Ground samples were scanned on a NIRSystems 5000 (Foss Pacific, North Ryde, NSW, Australia) spectrometer using a spinning module with round cells. Spectra were recorded and analysed using ISI Software (Foss Pacific). Spectral data (1108 – 2498 nm) were processed using SNV, Detrend with 1,5,5,1 or 2,5,5,1 settings and 2 outlier passes [10].

### 2.3 Isolation of soluble and insoluble fibre fractions

Samples of the grains and pulses were ground so that they passed through a 0.5 mm screen of a Tecator Cyclotec mill (Tecator AB, Hogenas, Sweden) and stored in air-tight containers at 4°C. At the time of grinding, a sample (1 – 2 g) was dried (105°C, 1 h) to determine the dry matter content for future calculations. Samples (about 200 mg) were accurately weighed and placed in 30 mL screw-capped culture tubes. A sequence of procedures were carried out to isolate soluble and insoluble fibre fractions. These procedures were based on the methodology of Theander and Westerlund [11] and Englyst and Hudson [12] and are described here only in outline; all the procedures were carried out in a single tube [13]. Fats were extracted from the samples using hexane. Free sugars were exacted using 80% aqueous ethanol at 80°C; this step also inactived endogenous hydrolytic enzymes. The residue was then treated with boiling sodium acetate buffer (pH 5) which extracted the soluble fibre fraction and gelatinized any starch granules. The residue was the insoluble fibre fraction. The starch in the soluble fibre fraction was hydrolysed to glucose by treating with a heat-stable α-amylase followed by an amyloglucosidase. An aliquot was taken to determine the starch content of the total sample (see Section 2.4) and then the soluble fibre fraction was precipitated by bringing the solution to 80% ethanol. For one of the NIR correlations, the content of neutral-detergent and crude fibre in the samples was determined [14].

### 2.4 Analysis of the polysaccharides in the fibre fractions

The starch content of the samples was determined by quantifying the glucose released (see Section 2.3) using a kit from Megazyme (Megazyme International, Bray, Ireland) according to the instructions available at www.megazyme.com. The kit is based on the method of Blakeney and Matheson [15] and uses a glucose oxidase/peroxidase glucose method stabilised by the use of *p*-hydroxbenzoic acid in the Trinder colour reaction. The β-glucans were determined using a Megazyme test kit which uses the specific enzymes and follows the method of McCleary and Glennie-Holmes [16] and McCleary and Codd [17]. Details of the method are available at www.megazyme.com. A review of the available methods for determining β-glucans [18] concluded that reliable estimates could be obtained only with methods

 

based on the use of the specific (1→3),(1→4)-β-glucan endo-hydrolase as recommended by Anderson *et al.* [19]. The non-starch polysaccharides (NSPs) in the fractions, including the β-glucans, were determined by analysing the monosaccharides released by acid hydrolysis. The polysaccharides in the soluble fibre fraction were hydrolysed using 2 M trifluoroacetic acid and in the insoluble fibre fraction using a two-stage sulphuric acid method [13]. The neutral monosaccharides were determined by a modification of the method of Blakeney *et al.* [4]; they were reduced, acetylated, and the resulting alditol acetates separated by capillary gas chromatography. The NSPs in the fractions were determined by summing the percentages of anhydro monosaccharides. The percentage of cellulose was calculated as the percentage of insoluble anhydro glucose minus the percentage of insoluble β-glucan. The percentage of arabinoxylan was calculated using Ara/Xyl ratios of typical cereal grain arabinoxylans.

## 3 Results and discussion

Table 1 shows the NIR calibration statistics for NSPs as measured in the grains and pulses. The coefficient of determination for cross validation (1-VR) is a measure of goodness of fit similar to $r^2$. For a 1-VR of 0.82, 82% of the variance in the NIR result can be accounted for by the sample reference analytical result. Partial least squares regression and cross-validation were used; cross-validation is a technique where each sample is, in turn, removed from the test set and used as a prediction sample. From a combination of the results the standard error of cross-validation can be computed. The RPD, which is the ratio of standard error of cross validation (SECV) and the standard deviation of the reference data, was used to evaluate the calibrations. Williams and Norris [7] developed a table based on RPD values that predicts the use of calibrations for classification of samples. Equations with RPD values > 3.1 are considered fair and suitable for screening samples. Those with RPDs > 5.0 are good and suitable for quality control applications, whereas those with RPDs > 6.5 are very good and suitable for analysis.

In the present study, NIR calibrations were attempted for the polysaccharides in both the soluble and insoluble fibre fractions (*e.g.*, arabinoxylan and β-glucan). Calibrations were also attempted for the percentages of individual monosaccharides (in their anhydro forms). Several of the calibration statistics appear promising in terms of RPD. For example, squared correlation coefficients ($r^2$) and standard errors of cross-validation (1-VR) for arabinoxylan in the soluble, insoluble and total fractions were 0.49, 0.18; 0.88, 1.03; and 0.88, 1.06, respectively, for approximately 70 diverse samples of grains and pulses. The apparently poor

**Table 1.** NIR statistics from ISI calibration software for NSPs in the total and fibre fractions of the samples of grains and pulses

| Component | *N* | Mean (%) | Range (%) | SD | 1-VR | SECV | RPD |
|---|---|---|---|---|---|---|---|
| *Insoluble fibre fraction* | | | | | | | |
| Insoluble arabinose[a)] | 70 | 2.5 | 1.2–3.20 | 0.7 | 0.82 | 0.31 | 2.26 |
| Insoluble xylose | 71 | 4.6 | 0.8–14.7 | 2.8 | 0.93 | 0.74 | 3.78 |
| Insoluble arabinose + xylose | 72 | 7.2 | 2.0–16.5 | 3.0 | 0.88 | 1.03 | 2.91 |
| Insoluble glucose | 71 | 4.2 | 1.8–11.4 | 2.3 | 0.81 | 1.00 | 2.30 |
| Total insoluble NSP | 71 | 10.9 | 3.9–25.2 | 4.9 | 0.89 | 1.59 | 3.08 |
| Insoluble arabinoxylan | 72 | 6.3 | 1.8–14.5 | 0.90 | 0.88 | 0.06 | 15 |
| Insoluble cellulose | 71 | 3.8 | 1.6–10.2 | 0.90 | 0.81 | 0.12 | 7.5 |
| Insoluble arabinoxylan + cellulose | 69 | 11.2 | 4.0–25.5 | 1.61 | 0.93 | 0.18 | 8.94 |
| *Soluble fibre fraction* | | | | | | | |
| Soluble arabinose | 73 | 0.21 | 0.04–0.40 | 0.10 | 0.59 | 0.06 | 1.67 |
| Soluble xylose | 75 | 0.24 | 0.01–0.90 | 0.17 | 0.50 | 0.12 | 1.41 |
| Soluble arabinose + xylose | 74 | 0.45 | 0.06–0.91 | 0.25 | 0.19 | 0.18 | 1.39 |
| Soluble glucose | 72 | 1.1 | 0.09–3.80 | 1.10 | 0.62 | 0.70 | 1.57 |
| *Total fibre* | | | | | | | |
| Total soluble NSP | 71 | 1.6 | 0.21–3.92 | 1.00 | 0.68 | 0.57 | 1.75 |
| Total arabinose | 71 | 2.7 | 1.20–5.5 | 0.78 | 0.84 | 0.31 | 2.51 |
| Total xylose | 71 | 4.8 | 0.70–14.8 | 2.80 | 0.93 | 0.76 | 3.68 |
| Total arabinose + xylose | 72 | 7.7 | 2.10–17.1 | 3.01 | 0.88 | 1.06 | 2.83 |
| Total β-glucan | 84 | 1.57 | 0.09–5.12 | 1.56 | 0.92 | 0.45 | 3.47 |

a) Anhydro monosaccharide

*N* = number of samples in calibration; SD = standard deviation; 1-VR = coefficients of determination for cross validation; SECV = standard error of cross validation.

RPD = SD/SECV

 

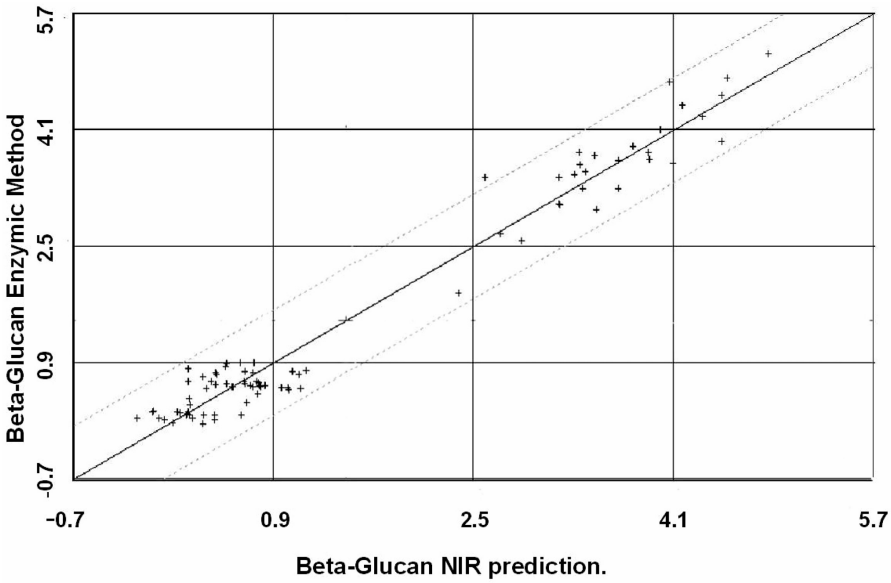


**Figure 1.** Plot showing the total β-glucan content of samples as reference and NIR predicted values. Barley grains are in the grop with >2.5% total β-glucan.

result for the soluble fraction is due to the narrow range of values encountered. However, the proportion of arabinoxylan in the soluble fraction is small in comparison with that in the insoluble fraction. The equations developed for insoluble arabinoxylan, cellulose, and insoluble NSPs are all very good and suitable for analytical use. The total β-glucan calibration is fair, but is divided into two populations (Fig. 1): barleys, which have >2.5% β-glucan, and other grains and pulses, which have a lower percentage of β-glucan. The number of samples available for the calibration was insufficient to split these groups and further samples would be needed to improve the calibration.

In other studies, not reported in detail here, 1-VR values of 0.84 for neutral detergent fibre with an SECV of 2.65 for all the grains and pulses (82) in the set were obtained. Crude fibre gave an even better 1-VR value of 0.95 for all grains and pulses with an SECV of 0.77, this dropped to 0.47 if only the cereal grains were included. The potential power of the analysis is shown by the results for the starch contents of the total samples (Table 2). These results gave a 1-VR of 0.94 and a SECV of 1.52 for the cereal grains. The minimum laboratory error for the determination of starch by modern enzymic methods is about 0.9%, and in interlaboratory tests this error often exceeds 2.0% [20]. The NIR result achieved here is thus comparable in accuracy to that obtained from the routinely used enzymic reference method.

The calibration statistics reported here indicate that NIR can be used in plant breeding, nutritional and product studies to obtain simple and rapid estimates of NSPs. It is well known that there are wheats with a high content of cell walls and with "woolly" milling characteristics, together with barleys with a high content of β-glucan [21]. However, to date, this genetic variation has not been extensively

**Table 2.** NIR statistics from ISI calibration software for the total starch content of the grains and pulses

| Total starch content | *N* | 1-VR | SECV |
|---|---|---|---|
| All grains and pulses | 82 | 0.76 | 3.34 |
| Cereal grains only | 72 | 0.94 | 1.52 |

exploited for the production of grains for use as human food ingredients. Genetic variation could be achieved either by having thicker cell walls, a larger number of smaller cells, or a combination of both. If definite nutritional advantage is perceived in growing cereal grains with altered concentrations of NSPs, it appears that both the natural genetic variation and the means of selection exist. This development will, however, be dependent on food processing companies developing suitable products and being prepared to pay the premiums for plant variety development and for grain production. NIR-based analysis of NSPs could provide a useful tool for the development and segregation of such grains.

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